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Giraudeau, M ; McGraw, K J

Abstract: Rapid worldwide urbanization is creating novel environments to which animals must adapt, a topic of growing interest for biologists. Studies of how organisms are affected by cities historically centered on large-scale censusing of populations, but recent investigations have considered finer-scaled, urban-rural differences among individuals and species in their behavior, morphology, and physiology, specifically as they relate to urban stress. A number of factors (e.g., corticosterone (CORT)-related stress response) may contribute to the degree of stress experienced by animals living under urban versus natural conditions, but several physiological variables have yet to be considered together in a large-scale assessment. Here, in a widespread species of desert passerine (the house finch, Haemorhous mexicanus), we quantified variation in plasma oxidative stress, plasma concentrations of vitamins and carotenoids, and body-mass of males in three successive seasons (winter, spring, and late summer/early fall) along an urban-rural gradient in Phoenix, Arizona, USA. We found that degree of urbanization was: (1) negatively related to circulating vitamin A concentrations in winter, (2) positively correlated with body-mass during spring, and (3) negatively associated with plasma concentrations of two carotenoids: zeaxanthin (during breeding) and 3-hydroxy-echinenone (3HE) (during molting). The striking link between 3HE levels and urbanization is consistent with previous research showing that urban songbirds have lower carotenoid levels and faded plumage; our finding is the first to implicate specific effects on a metabolically derived carotenoid for coloration. The fact that we observed only season-specific links between urbanization and indicators of quality in finches suggests that (at least for these metrics) there are no strong, lasting urban pressures imposed on finch physiology over the year. Interestingly, we found that a metric of plasma oxidative stress (lipid peroxidation) was positively correlated with levels of two carotenoids (lutein during breeding and 3HE during molting), which is consistent with a prior study of ours showing that finches with redder plumage deposit higher levels of CORT in their feathers; taken together, our studies suggest complex associations between carotenoids and stress

DOI: https://doi.org/10.1093/icb/icu024

Integrative and Comparative Biology

Integrative and Comparative Biology, volume 54, number 4, pp. 622–632
doi:10.1093/icb/icu024

Society for Integrative and Comparative Biology

SYMPOSIUM

Physiological Correlates of Urbanization in a Desert Songbird

Mathieu Giraudeau[1,*,†] and Kevin J. McGraw*

*School of Life Sciences, Arizona State University, Tempe, USA; †Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

From the symposium "Stress, Condition and Ornamentation" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

[1]E-mail: giraudeau.mathieu@gmail.com

Synopsis Rapid worldwide urbanization is creating novel environments to which animals must adapt, a topic of growing interest for biologists. Studies of how organisms are affected by cities historically centered on large-scale censusing of populations, but recent investigations have considered finer-scaled, urban–rural differences among individuals and species in their behavior, morphology, and physiology, specifically as they relate to urban stress. A number of factors (e.g., corticosterone (CORT)-related stress response) may contribute to the degree of stress experienced by animals living under urban versus natural conditions, but several physiological variables have yet to be considered together in a large-scale assessment. Here, in a widespread species of desert passerine (the house finch, *Haemorhous mexicanus*), we quantified variation in plasma oxidative stress, plasma concentrations of vitamins and carotenoids, and body-mass of males in three successive seasons (winter, spring, and late summer/early fall) along an urban–rural gradient in Phoenix, Arizona, USA. We found that degree of urbanization was: (1) negatively related to circulating vitamin A concentrations in winter, (2) positively correlated with body-mass during spring, and (3) negatively associated with plasma concentrations of two carotenoids: zeaxanthin (during breeding) and 3-hydroxy-echinenone (3HE) (during molting). The striking link between 3HE levels and urbanization is consistent with previous research showing that urban songbirds have lower carotenoid levels and faded plumage; our finding is the first to implicate specific effects on a metabolically derived carotenoid for coloration. The fact that we observed only season-specific links between urbanization and indicators of quality in finches suggests that (at least for these metrics) there are no strong, lasting urban pressures imposed on finch physiology over the year. Interestingly, we found that a metric of plasma oxidative stress (lipid peroxidation) was positively correlated with levels of two carotenoids (lutein during breeding and 3HE during molting), which is consistent with a prior study of ours showing that finches with redder plumage deposit higher levels of CORT in their feathers; taken together, our studies suggest complex associations between carotenoids and stress.

Introduction

Urbanization has accelerated worldwide in recent decades, and now more than half of the human population lives in cities (Crane and Kinzig 2005). This dramatic expansion of urban centers has come at the detriment of natural environments and putatively of the animals that inhabit them. Anthropogenic change in land-use is argued to impact species distributions and richness over the long term (Marzluff et al. 2001; Shochat et al. 2004; Rubbo and Kiesecker 2005; McKinney 2008), via its shorter-term effects on survival and reproduction (Ryder et al. 2010; Fischer et al. 2012; Seress et al. 2012).

Identifying precisely which components of fitness are constrained (or facilitated in some cases) by urbanization has been a challenge tackled by organismal biologists in recent years (McCleery 2009; Deviche and Davies 2013; Martin and Boruta 2013). Among the various attributes facilitating survival of wild animals, the role of stress has received considerable attention (French et al. 2008; Bonier 2012; Steinberg 2012). Many anthropogenic changes to the environment, be it construction of buildings or the mere presence of more humans, can serve as potent stressors that lead animals to avoid urban areas or suffer by living in cities (Bonier et al. 2007; French et al. 2008;

Advanced Access publication May 8, 2014

Fokidis et al. 2009). Stress can take many different physiological and behavioral forms, and most commonly stress has been parameterized in urban contexts as a function of the corticosterone/cortisol (CORT)-mediated stress response (Bonier 2012). The majority of the studies examining urban variation in CORT have been performed on birds, showing no consistent pattern among species and/or geographical locations (Partecke et al. 2006; Bonier et al. 2007; Schoech et al. 2007; French et al. 2008; Fokidis et al. 2009; Bonier 2012). More studies are needed, however, to consider the myriad other causes and manifestations of stress (e.g., nutritional, predator-induced) in this urban context, and consider them simultaneously so as to obtain a more comprehensive understanding of the stress-related impacts of cities on free-ranging organisms.

Recently, ecological studies of oxidative stress have been on the rise (McGraw et al. 2010a). This line of work has emerged from observations that antioxidant/oxidant balance is critical for somatic maintenance and other key life-history features (Costantini et al. 2010), including sexual signaling (Lozano 1994; von Schantz et al. 1999). A few studies have extended this into urban systems; for example, the expression of sexually selected carotenoid coloration is reduced in some urban populations of birds (Hõrak et al. 2001; Jones et al. 2010), and in these instances both the dietary supply of carotenoids may be depleted (Isaksson and Andersson 2007) and oxidative stress elevated (Isaksson et al. 2005). Fitness consequences of oxidative stress in cities, however, have yet to be uncovered, as are links to other key physiological parameters. Carotenoids are argued to be weak antioxidants in some wild animals, for example (Costantini and Møller 2008), and other antioxidants, such as the lipid-soluble vitamins (A-retinol and E-tocopherol), may be more vital for combating oxidative stress (Hartley and Kennedy 2004). Only one study to date has considered vitamin profiles within the context of an urban environment, and Møller et al. (2010) found that rural populations of birds had higher levels of circulating vitamin E (and total plasma carotenoids) than did urban birds from the same species.

Here, we report on a study of several biomarkers of organismal physiology in wild animals along a gradient of urbanization (Bokony et al. 2012; Giraudeau et al. 2014). Specifically, we investigated variation in plasma levels of vitamin A, vitamin E, and carotenoids, as well as of one measure of oxidative stress (lipid peroxidation; see more below). We also examined urban–rural differences in body-mass, which may serve as a more integrated indicator of somatic quality. We studied all of this in house finches (*Haemorhous mexicanus*), a widespread species of songbird in North America that is native to southwestern desert habitats but also inhabits human environs (Hill 1993). House finches have been a popular model for the study of carotenoids and male plumage ornamentation (Hill 2002; McGraw et al. 2006a), and we have previously shown in our study populations in Phoenix, AZ, USA that urban male finches display drabber plumage (authors' unpublished data) and harbor more gastrointestinal parasites (coccidians) and viral pathogens (poxvirus) (Giraudeau et al. 2014) than do rural males. We conducted the present study of body mass and plasma antioxidants and oxidative stress at the eight prior study sites along an urban gradient (from Giraudeau et al. 2014). This design permitted careful comparison of finch biometrics to quantitative urban parameters (e.g., human population density, land-use characteristics; see more below), as opposed to simply comparing traits between single urban versus rural populations, as occurs in many such studies. Moreover, to assess potential seasonal shifts in the links between physiology and urbanization, we gathered data from finches at three different periods in the year (winter non-breeding, spring breeding, and late summer/early autumn molt). Based on our prior work (Giraudeau et al. 2014), and that of others showing that parasites can both depress levels of carotenoids and lipid-soluble nutrients (Thompson et al. 1997; Hõrak et al. 2004) and elevate oxidative stress (Costantini and Møller 2009; Sorci and Faivre 2009), we predicted that circulating vitamins and carotenoids would decrease in more urbanized areas. Although we previously found no link between oxidative stress and urbanization in an autumn sample of birds from our study populations (Giraudeau et al. 2014), here we also tested whether or not our measure of oxidative stress would increase in more urbanized areas in this longer-term investigation. We also examined seasonal changes in finch physiological parameters, and how carotenoids with different roles (e.g., red ketocarotenoids important for attractive plumage color development; McGraw et al. 2006a) may vary differently with season and degree of urbanization.

Methods

Field methods

From January 6–September 15, 2011, we used basket traps and Potter traps baited with sunflower seeds (Giraudeau et al. 2012) to capture 360 male house finches from eight sites along a gradient of

Table 1. Data gathered on degree of urbanization for each of our eight study sites in the Phoenix, AZ, USA metropolitan area. See "Methods" section for further details on the sources of this information and how human-population data and land-use characteristics were used to determine degree of urbanization

Capture Site	Phoenix Downtown	ASU Campus	Mesa Organic Farm	Crossroads District Park	Chandler Neighborhood	Phoenix Zoo	South Mountain Park	Estrella Mountain Park
City	Phoenix	Tempe	Mesa	Gilbert	Chandler	Phoenix	Tempe	Goodyear
Geographical coordinates	33°27′N 112°03′W	33°25′N 111°55′W	33°27″N 111°49′W	33°19′N 111°43′W	33°18′N 111°55′W	33°27′N 111°57′W	33°21′N 112°4′W	33°25′N 112°25′W
Human population living within 1 km of study site	7291	10,385	4600	17,175	3948	50	1001	11
Sample size in winter, spring, and fall	16, 17, 14	16, 19, 13	0, 18, 11	19, 14, 9	13, 11, 18	17, 15, 15	26, 17, 15	18, 12, 17
Habitats (% of land covered)								
Building	13.70	20.08	8.55	10.51	18.57	3.31	4.70	0.04
Road	37.02	30.82	21.72	18.12	23.11	21.66	7.32	3.02
Soil	37.31	19.33	47.17	41.03	21.59	42.36	69.89	72.41
Tree	4.82	14.48	8.80	8.61	22.49	8.33	1.41	1.06
Grass	7.10	6.94	10.48	17.25	13.31	14.76	1.93	0.20
Shrub	0	0	0.004	0	0	7.56	12.63	18.58
Cropland	0	0	0.12	2.49	0	0	0	0
Lake	0	0.0009	2.19	1.55	0.18	1.51	0	0.10
Canal	0	0.14	0.67	0.33	0	0.50	0	0
Pool	0.05	0.21	0.19	0.10	0.74	0.01	0.03	0.02
River	0	0	0.07	0	0	0	2.09	4.58

urbanization in the Phoenix metropolitan area (see Table 1 for the samples sizes per site and season). These sites varied in distance from 1 to 35 km to the urban Phoenix center and in many other environmental aspects (Table 1). We focused on males because of our interest in finch carotenoid physiology and coloration. We restricted capture to a few days per season to avoid additional, intraseasonal temporal variation in our finch parameters, though we cannot address how larger-scale urban–rural temporal variability in factors like breeding onset (Partecke et al. 2004) may have impacted our results. Within 20 min of capture, each bird was weighed (to the nearest 0.1 g) and leg-banded with a numbered United States Geological Survey metal tag for individual identification. We also collected 150 µl of whole blood through the alar vein with heparinized capillary tubes. Blood was centrifuged (10,000 rpm for 3 min) and the plasma saved at −80°C for later analysis of oxidative stress (lipid peroxidation) and circulating carotenoids and vitamins. Some of the 360 birds were recaptured and sampled multiple times during our study, but they only appear once in our dataset for statistical analyses; in these instances, we used the data from the sampling point at which we had the most complete physiological information (i.e., no missing body mass or plasma data).

Measurement of plasma carotenoids, vitamins, and oxidative stress

We followed prior methods for carotenoid extraction and analysis via high-performance liquid chromatography (HPLC) (McGraw et al. 2013), which included (unlike in our other prior study of finch plasma carotenoids; McGraw et al. 2006a) the additional acid pre-treatment of our HPLC column (Toomey and McGraw 2007) that permitted us to recover ketocarotenoids if present. Among the 338 total plasma samples (winter 2011: $n = 115$; spring 2011: $n = 118$; summer/fall 2011: $n = 105$), we detected vitamin A (retinol; Navarro et al. 2010), vitamin E (alpha-tocopherol; Giraudeau et al. 2013), and seven different types of carotenoids: lutein, zeaxanthin, β-cryptoxanthin, β-carotene, dehydrolutein, 3-hydroxy-echinenone (3HE), and an esterified form of 3HE (Fig. 1). Based on prior work, we presume that the first four carotenoids are dietary in origin and the latter three are metabolically derived forms (Inouye et al. 2001). We were especially interested in

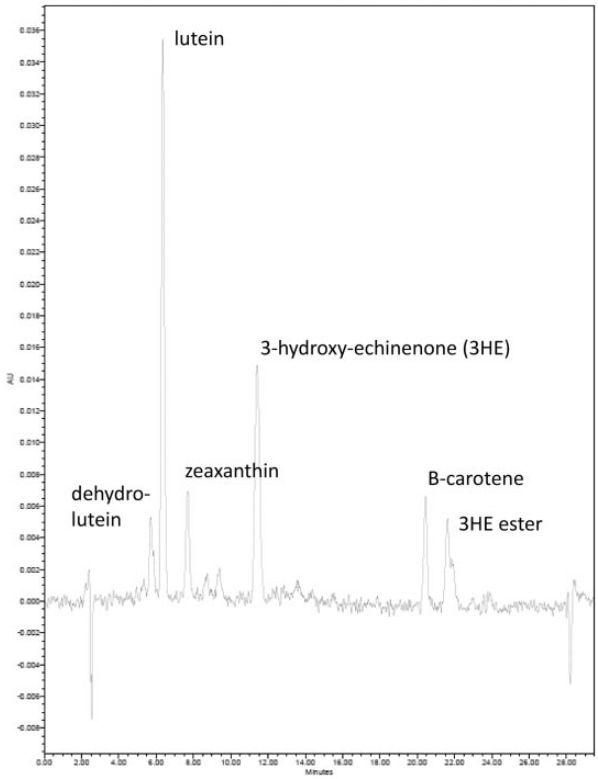

Fig. 1 Two-dimensional HPLC chromatogram depicting the carotenoids detected in plasma of house finches.

3HE because this is the primary ketocarotenoid pigment determining red coloration in the plumage of male house finches (Inouye et al. 2001). Due to the strong intercorrelation between the free and esterified forms of 3HE ($r^2 = 0.80$, $n = 99$, $P < 0.0001$), we report and analyze it here as total 3HE. β-Cryptoxanthin was absent in 294 of the samples and was detected above baseline levels in only eight total samples, so we do not statistically analyze it here. Methods for analyzing plasma oxidative stress (i.e., lipid peroxidation, using the thiobarbituric acid-reactive substances [TBARS] test) follow those described in Giraudeau et al. (2013, 2014); note that the summer/fall 2011 TBARS dataset is the same here as in Giraudeau et al. (2014).

Metrics of urbanization

To assess relationships between finch physiological metrics and anthropogenic environmental characteristics, we obtained several urbanization parameters around our eight trapping sites from a local database that is part of the Central-Arizona-Phoenix Long Term Ecological Research program (Stefanov et al. 2001, 2007; Stefanov and Netzband 2005): (1) resident human population density within a radius of 1 km around each trapping site, estimated from the 2010 US Census data; (2) landuse and landcover variables in 2010 within the same 1 km radius. We selected 1 km radii because this is the range within which we re-capture and re-sight adult finches relative to their original capture/banding site (personal observation). From satellite images taken in 2010 as part of the National Agriculture Imagery Program, we determined percentage of land dedicated to 11 land uses: building, road, soil, tree, grass, shrub, cropland, lake, canal, pool and seasonal river (Stefanov et al. 2001, 2007; Stefanov and Netzband 2005). Using principal component analysis (PCA), urbanization scores were generated using the data for the 12 variables cited above (11 land-use variables and human population density). PCA indicated that two PCs captured >75% of habitat variation. PC1 summarized 51.2% of the variance (eigenvalue = 6.14), whereas PC2 summarized 23.9% of the variance (eigenvalue = 2.87). PC1 strongly correlated positively with human population density and the percentage of land covered by buildings, trees, and grass, and negatively with the percentage of land covered by shrubs, river, and soil (Table 2); thus, we interpret higher PC1 scores as more urbanized and developed. PC2 strongly correlated positively with the percentage of land covered by lake and canal, so sites with higher PC2 scores have more artificial water supplies (Table 2).

Statistics

We used Pearson's correlational analyses to compare our PC1 and PC2 metrics of urbanization with population means for our plasma variables (oxidative stress, vitamins, and carotenoids) and for body mass for each season. All statistical analyses were carried out using Statview 5.0.1 (SAS Institute Inc., Cary, NC, USA) with α set at 0.05.

Results

Overall and annual patterns of body mass and plasma carotenoid, vitamin, and oxidative levels

Across all samples, lutein was the most concentrated carotenoid in plasma (Fig. 2A), except during the summer/fall (molt) period when 3HE was most concentrated. DHL and zeaxanthin were next most concentrated, followed by β-carotene and (as mentioned above) β-cryptoxanthin. Circulating levels of lutein, zeaxanthin, and dehydrolutein declined across the study, whereas β-carotene peaked in spring (being absent in all but one sample during summer/fall) and 3HE was present only in summer/fall (but in 99/105 total samples during that time of year). Vitamins A and E were generally present in low concentration in plasma; both declined in concentration

Table 2. Factor loading matrix for principal components 1 and 2, which serve as our metrics of urbanization in this study. Loadings in bold are >0.4

Variable	PC1	PC2
Population density	**0.64**	0.26
% Buildings	**0.85**	**−0.48**
% Roads	**0.78**	−0.25
% Soil	**−0.94**	0.26
% Tree	**0.81**	−0.32
% Grass	**0.76**	**0.50**
% Shrub	**−0.96**	−0.06
% Crop	0.29	**0.61**
% Lake	0.25	**0.89**
% Canal	0.27	**0.81**
% Pool	**0.58**	**−0.47**
% River	**−0.91**	−0.17

from winter to spring, with vitamin A rising in summer/fall while vitamin E remained unchanged (Fig. 2B). Similar to vitamin A, lipid peroxidation levels were higher in winter and summer/fall than in spring, whereas body mass declined across the year (Fig. 2C).

Links to urbanization—Winter 2011 samples

We found that circulating vitamin A levels were significantly negatively correlated with urban PC1 (Fig. 3), meaning that concentrations were higher in rural finches. Plasma vitamin E and β-carotene levels were also strongly negatively associated ($r^2 = 0.874$, $P < 0.0001$; all other $P > 0.085$).

Links to urbanization—Spring 2011 samples

Urban PC2 was negatively correlated with zeaxanthin levels and positively correlated with body mass (Fig. 4), meaning that birds from sites with more artificial water had lower zeaxanthin levels but weighed more. Plasma vitamin E concentration again was strongly negatively correlated with β-carotene levels ($r^2 = 0.651$, $P = 0.012$) as well as with lutein levels ($r^2 = 0.602$, $P = 0.201$) during this season. Also, lutein concentrations were positively correlated with dehydrolutein levels ($r^2 = 0.679$, $P = 0.009$) and with lipid peroxidation levels ($r^2 = 0.517$, $P = 0.043$; all other $P > 0.06$).

Links to urbanization—late summer/early autumn 2011 samples

Plasma concentrations of 3HE were highly significantly negatively correlated with urban PC1

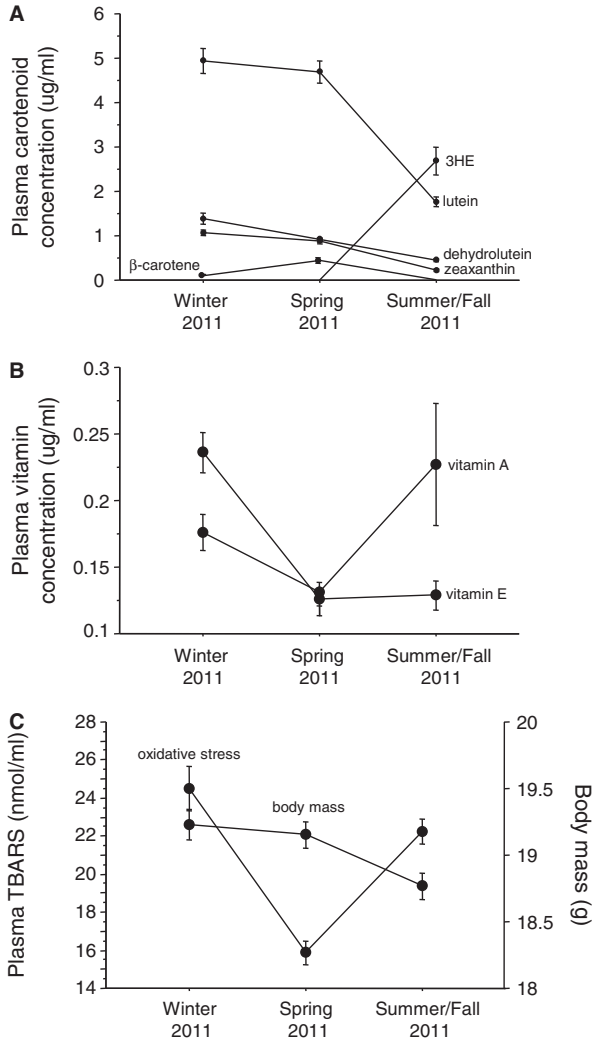

Fig. 2 Seasonal variation in (**A**) plasma carotenoid concentrations, (**B**) plasma vitamin A and E concentrations, and (**C**) body mass and oxidative stress in winter, spring, and autumn 2011. Population means and standard error are depicted.

(Fig. 5), such that rural males circulated higher levels. In fact, 93% of population-level variation in 3HE concentration was accounted for by our first principal component of urbanization. As in the breeding season, lutein and dehydrolutein levels were strongly positively correlated ($r^2 = 0.958$, $P < 0.0001$). Interestingly, we found that lipid peroxidation levels were positively correlated with 3HE concentrations during the molt period (Fig. 6; all other $P > 0.06$).

Discussion

Annual variation in physiological parameters

In each of three seasons during the year (winter, spring, and late summer/early fall), we surveyed body mass and plasma levels of carotenoids,

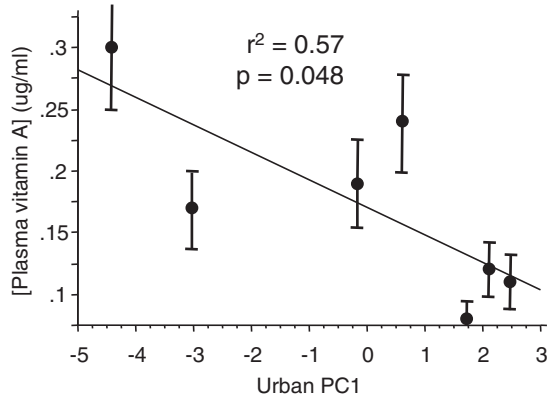

Fig. 3 Relationship between urbanization PC1 scores and mean plasma vitamin A concentration at each of our seven study sites for which we gathered data on house finch physiology in winter 2011. Here and in subsequent figures, error bars depict population standard errors.

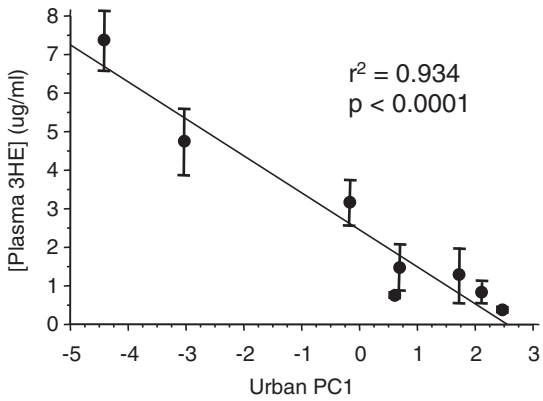

Fig. 5 Relationship between urbanization PC1 scores and average plasma 3HE concentration at each of our eight study sites for which we gathered data on house finch physiology in summer/fall 2011.

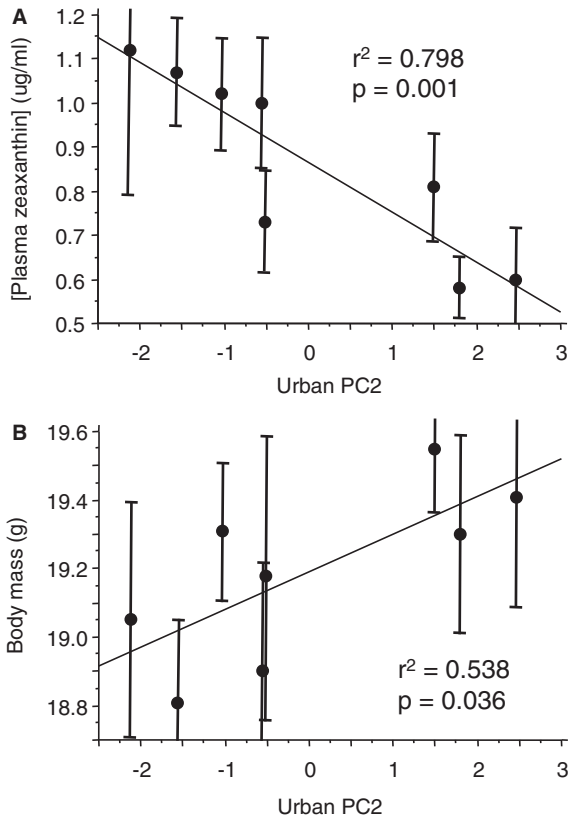

Fig. 4 Relationship between urbanization PC2 scores and (**A**) average plasma zeaxanthin concentration, (**B**) average body mass at each of our eight study sites for which we gathered data on house finch physiology and morphology in spring 2011.

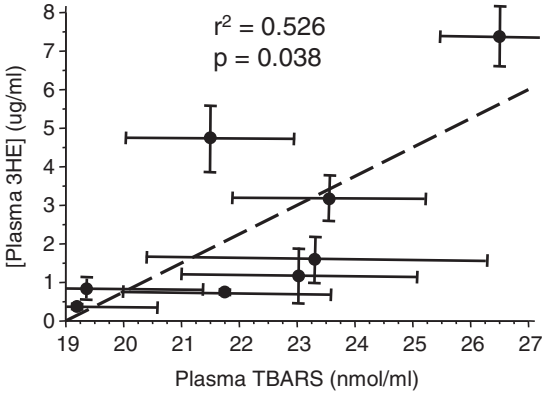

Fig. 6 Relationship between average plasma TBARS levels and average plasma 3-hydroxy-echinenone concentration at each of our eight study sites for which we gathered data on house finch physiology in summer/fall 2011.

lipid-soluble vitamins, and lipid peroxidation (a marker of oxidative stress) in male house finches captured at eight different sites along a gradient of urbanization. Across all samples, we found that body mass declined across the year, as might be expected as these finches progressed through challenging times (e.g., onset of breeding, then an extraordinarily hot summer followed by an energetically expensive plumage molt; O'Connor 1995; Swaddle and Witter 1997). Levels of lipid peroxidation and plasma vitamins also declined from winter to spring, but unexpectedly both rebounded in the late summer/early fall (though vitamin E remained constant from spring to late summer/early fall). This indicates that antioxidant/oxidative physiology is uncoupled from body mass during molt and closely tracks current antioxidant nutrition and/or oxidative challenges. Lush vegetation (and thus food availability for these herbivores) declines from spring breeding until the monsoon season (July–August), when rains bring abundant foraging opportunities concurrent with the molt season. Hence, we might expect a

rise in many nutrients (including vitamins) at this time, though apparently this is not sufficient to counteract the oxidative crises that occurred in late summer/early fall (Vieira and Gomes 2010).

Similar to body mass, we found that levels of three circulating carotenoids (lutein, zeaxanthin, and dehydrolutein) declined from winter to spring to late summer/early fall. Lutein and zeaxanthin are the two most widespread xanthophylls in herbivorous bird diets (McGraw et al. 2001; McGraw 2006), and dehydrolutein is purportedly a lutein derivative (i.e., given its close correspondence with lutein in our study; McGraw et al. 2003), so this annual drop certainly may be attributed in part to dietary availability. However, these carotenoids may be physiologically depleted from the body due to oxidative challenges during breeding and molt (i.e., if serving as antioxidants; Krinsky 1989; Lozano 1994; von Schantz et al. 1999), and specific to molt can be deposited into plumage (Inouye et al. 2001) or serve as precursors for other metabolically produced carotenoids (McGraw et al. 2006a). In contrast, the one ketocarotenoid we detected in circulation—3HE—was present in circulation only during late summer/early fall, and this signifies the seasonally timed, specialized process of manufacturing red pigments only when new ornamental plumage is being grown (McGraw et al. 2013).

Physiological variation along the urban gradient

When we considered variation in physiological parameters along our gradient of urbanization, five main patterns emerged. First, circulating vitamin A concentrations were associated with degree of urbanization (PC1) in winter, such that birds circulated higher levels of vitamin A in areas with fewer buildings. Effects of pollutants on vitamin A have been shown in laboratory animals and in several wild species (Simms et al. 2000; Jenssen et al. 2003; Braathen et al. 2004; Debier et al. 2005). More specifically, some pollutants (e.g., PHAH and PCB) can enhance the catabolism of vitamin A and its clearance from the liver (Bank et al. 1989; Roberts et al. 1992; Zile 1992; Rolland 2000). Thus, exposure to urban pollutants may have affected vitamin A levels in our study, and this makes sense given that anthropogenic air pollution is highest during winter in Phoenix (Smith et al. 2000). Alternatively, dietary availability of vitamin A precursors may decrease in urban sites in winter. Finches in large numbers come to rely on feeder foods at this time of year (personal observation), and these are deficient in the primary vitamin A precursor for herbivores (β-carotene; McGraw et al. 2001). Interestingly, we also found circulating β-carotene levels to be comparatively low in finches during winter.

Second, degree of urbanization (PC2) was positively associated with body mass and negatively associated with circulating zeaxanthin levels in spring, meaning that birds were heavier but circulated less zeaxanthin in areas with more artificial water bodies. Previous studies have found that house sparrows (Passer domesticus) have reduced body mass in urbanized habitats (Liker et al. 2008), though no consistent relationship between urbanization and the scaled mass index was detected (Bokony et al. 2012), showing that urban house sparrows are smaller but not leaner than rural ones. In our case, the relationships between PC2 and body mass/zeaxanthin may be explained by the food provided by humans in parts of the Phoenix metro area. Seeds from bird feeders (e.g., sunflower and millet) provide a reliable source of calories and fat but are poor in carotenoids (McGraw et al. 2001) in suburban areas with large artificial lakes (Hensley 1954; Mills et al. 1989; Hill 1993; Shochat et al. 2004). Thus, birds in developed areas that feed heavily from feeders have abundant access to a concentrated food source, perhaps reducing energy expended on foraging trips and thereby fostering large body mass, while also leading to reduced levels of a dietary xanthophyll like zeaxanthin. This may especially have been true during the spring breeding period, as this biparental species must forage for both self and many offspring (they can produce up to three successful nests and 12–14 offspring per year; Hill 1993).

Thirdly, we showed that degree of urbanization was negatively associated with circulating levels of 3HE during late summer/early fall. This carotenoid type is the most abundant red pigment in the plumage of male house finches (Inouye et al. 2001). In fact, our discovery that 3HE was not present in circulation at any time of year other than during fall indicates that this process is incredibly seasonal and/or physiological-state specific to molt. Previously we have found that the plumage of male house finches is redder in rural areas (authors' unpublished data), and our findings here for 3HE seem to account for the pigment-specific mechanism underlying this color pattern. This red pigment is produced through a metabolic transformation (putatively synthesized in the liver; del Val et al. 2009) of other dietary forms of carotenoids (Inouye et al. 2001), and, since we found no urban–rural differences in dietary carotenoids during the molt period, we suspect that urban stressors are directly impacting enzyme-driven metabolic conversion efficiency. Before this hypothesis

can be tested, more information is needed on the site(s), origin, and production mechanisms underlying 3HE accumulation.

Fourth, we found that plasma lipid peroxidation levels were positively associated with circulating levels of two carotenoids (lutein in spring and 3HE in fall). Typically, it is argued that antioxidant carotenoids should reduce oxidative stress (Krinsky 1989; Lozano 1994; von Schantz et al. 1999), but there is little evidence that carotenoids are strong antioxidants in birds (Isaksson and Andersson 2008; Costantini and Møller 2008) and in fact it has been argued that carotenoids can serve as prooxidants under certain conditions (Huggins et al. 2010). In one of our study populations, we previously showed in molting male house finches that plumage redness is positively associated with another form of stress—concentrations of corticosterone in feathers (Lendvai et al. 2013). From all of this, we find little evidence for the traditional health-enhancing role of carotenoids in house finches and must look to alternative mechanisms for explanation. One possibility is that a rise in the sex hormone testosterone (which circulates at higher levels in redder male house finches; Duckworth et al. 2004) may be responsible both for increased carotenoid circulation (Blas et al. 2006; McGraw et al. 2006b) and for elevated oxidative stress (i.e., the oxidation handicap hypothesis; Alonso-Alvarez et al. 2007). Future studies should examine how circulating levels of testosterone vary along the gradient of urbanization in house finches and may impose an oxidative cost on more carotenoid-rich birds.

Fifth, as in our previous study (Giraudeau et al. 2014), we did not find a significant correlation between degree of urbanization and lipid peroxidation levels in any season (only a tendency was found in fall; $P = 0.07$). These results confirm those obtained by Isaksson et al. (2009), who did not find any differences of lipid peroxidation, also measured with the TBARS assay, in lungs between urban and rural adult great tits. Similarly, rural and urban male frogs (*Rana ridibunda*) had similar levels of an oxidative stress index integrating several markers of oxidative stress in the Ukraine (Falfushinska et al. 2008). These results seem surprising in light of a few studies in animals (great tits, *Parus major*: Isaksson et al. 2005; house sparrow Herrera-Duenas et al. 2014) and in numerous studies of humans (mostly studying the effects of urban air pollution) that have found lower oxidative stress in rural compared with urban populations (Hong et al. 2009; Bono et al. 2013). However, there are many different biomarkers for oxidative stress (Sepp et al. 2012), and we must carefully consider which of these are employed as we learn more about urban impacts on physiological stress in free-ranging organisms.

In sum, we have found in house finches that several physiological parameters, many of which are key to their fitness (e.g., health and coloration; McGraw et al. 2010b; Toomey and McGraw 2012), vary as a function of degree of urbanization in the native desert southwestern range of this species. Many of these links were season-specific, which suggests that we should view the urban landscape not as one that homogenizes an environment and universally imposes static selective pressures over a year, but one whose abiotic and biotic influences are dynamic both in space and time. As has been done with some physiological parameters like CORT stress (Partecke et al. 2006), common-garden laboratory experiments are now in order to better ascertain whether these urban physiological variations are environmentally latent/plastic or longer-lasting due to genetic or developmental underpinnings.

Acknowledgment

We thank the editor and two anonymous referees for their constructive comments on the article. We are grateful to Michael Lundgren, Ron Rutowski, the staffs of the Love grows farm, Phoenix zoo, the city of Gilbert, the South Mountain and Estrella mountain regional parks for providing facilities during field work; and to Lucas Cousino, Erick Lundgren, Jodi Smith, Ryan Fountain, Alyssa Moore for assistance in data collection. We thank Chelsie Bruning Mateosky and Autumn Moore-Barkus for help with the HPLC analysis.

Funding

This work was supported by the National Science Foundation (IOS-0923694 to K.J.M.; and CAP3: BCS-1026865 to the CAP-LTER program), and a Fonds zur Förderung des akademischen Nachwuchses (FAN) grant (to M.G. during manuscript preparation).

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